IN VITRO REGENERATION OF BASELLA ALBA L.

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ABSTRACT

Basella alba L. is a tropical vine used as a vegetable in some Asian and African countries. It has potential as a non-traditional crop for small family farms. A short day plant, it blooms during fall, provided the temperatures are mild. In the southeastern United States, the short days of fall are associated with sub-freezing temperatures, and plants are killed before blooming. In this study attempts were made to regenerate the plant using tissue culture techniques. Several trials were conducted with different media, hormones and explants. It was found that nodal segments on Gamborg medium regenerated shoots. Interaction studies of auxins and cytokinins indicated that its endogenous auxin content might be high because callus proliferated in almost all treatments and roots initiated even when the medium was not supplemented with an auxin.

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Introduction

In recent years an increasing interest has been expressed in non-traditional crops that show promise for small American family farms (Tiwari et. al., 1988). Basella alba could be a promising new vegetable crop for small farms because it is pest and disease resistant, it has a high nutritive value, and its yield is 8 times higher than regular spinach (Yamaguchi, 1983).

Basella alba L. is a tropical vine (Bailey, 1951). It is used as a vegetable in some countries of Southeast Asia (Yamaguchi, 1983 and Tiwari et. al., 1988). Basella leaves and stems taste like spinach; therefore, it is known as "Malabar Spinach" in English (Yamaguchi, 1983). In India and Bangladesh it is known as "Poi Sag" (Tiwari et. al., 1988). In China it is called "Lok Kwa" (Yamaguchi, 1983).

Basella alba L. blooms during the short days of fall provided the temperatures are mild at nights. In Mississippi and all other parts of the southeastern United States short days are associated with sub-freezing temperatures at night. Therefore, Basella alba L. vines die before flowering.

Plant tissue culture techniques have been valuable in alleviating such problems through vegetative propagation of plants (Evans et. al. 1983). Plant regeneration in vitro is the key to practical application for most purposes using tissue culture techniques (Tomes, 1985).

Regeneration of any member of Basella alba L. has not been reported with the use of tissue culture techniques. Therefore, the purpose of this study was to investigate regeneration of Basella alba L. in vitro.

Materials and Methods

This study was conducted in three phases. The objective of phase I was to compare the performance of the three most used plant tissue culture media: Murashige & Skoog (MS), Anderson (AND) and Gamborg B5 (GB5) on callus proliferation, and root and shoot initiation on five explants: petiole, stem, meristem, leaf and node. Explant used were 0.5 cm^3 in size or segments of stem, leaf, petiole, meristem and node. All of the explants were put in Murashige and Skoog (MS), Anderson (AND) and Gamborg B5 (GB5) media after surface sterilization. Explants were sterilized as described by Thorpe (1981). MS medium had 100, 0.5, 0.5, 0.1, 2.0 and 80.0 mg/l of myo-inositol, nicotinic acid, pyrodoxine HCl, thymine HCl, glycine and adenine sulfate (Hartman and Kester, 1983). AND medium was supplemented with 4 mg/l thiamine HCl, 100 mg/l myo-inositol and 80 mg/l adenine sulfate (Hartman and Kester, 1983). GB5 medium had 10.0, 1.0, 100.0 and 80.0 mg/l of thiamine HCl, nicotinic acid, pyridoxine HCl, myo-inositol and adenine sulfate respectively in addition to basal inorganic salts (Hartman and Kester, 1983). Growth regulators for callus proliferation, shoot and root initiation trials were 0.1, 0.2, 0.4, 0.8 and 1.6 mg/l of napthaleneacetic acid (NAA) as auxin and 0.1, 0.2, 0.4, 0.8 and 1.6 mg/l of benzyl adenine (BA) as cytokinin. In every trial, one set without growth regulators was used as control. The pH of the media were adjusted to 5.7 before autoclaving. All three culture media had 30 g/l sucrose and 7 g/l agar (Thorpe, 1983). Media were autoclaved at 121°C for 20 minutes. The culture environment was maintained at 25°C and illuminated with cool white fluorescent lamps at 40 u

Einsteins/m^2/sec for 16 hours per day. After 3 weeks the cultures were evaluated by determining the size of calluses and total number of shoots greater than 5 mm in length produced per explant.

The objective of the phase II experiments was to evaluate the interactions of napthalenenacetic acid (NAA) and benzyl adenenine (BA) on callus proliferation and initiation of roots and shoots from explants selected earlier in phase I. It was 6 x 6 diallel experiment (Sondahl et. al., 1981). Dilution of NAA and BA were 0, 0.1, 0.2, 0.4, 0.8 and 1.6 ppm. Each treatment had 5 replications.

Mature vines were used as source of explants. The explants used were 3 - 5 mm segments of nodes. The explants were surface sterilized with 20% chlorox for 5 minutes and washed with distilled water 5 times. (Thorpe, 1981). Standard Gamborg B-5 medium was used as a source of nutrition for explant growth. It was supplemented with 3% sugar and 0.8% agar. After addition of NAA and BA, the pH of the medium was adjusted to 5.7 - 5.8 by NaOH and/or HCl solution. The medium was sterilized and autoclaved at 121°C at 15 lbs. PSI for 15 minutes.

Results and Discussion

Five explants: leaf, stem, meristem, petiole and nodal segments, and three media: MS, AND and GB-5 were used in phase I experiments. It was found that all explants initiated calluses but shoot initiation was restricted to a few combinations. Leaf explants on all three media showed signs of cell growth and cell elongation but did not produce a well defined callus. Stem, meristem and petiole initiated calluses on all three media are under investigation. Nodes produced calluses and proliferated shoots on GB5 and AND media but not on MS medium. In primary cultures shoot initiation occured only with the use of GB5 and AND media. Leaf, stem, meristem and petiole when used on shooting medium initiated calluses. The failure of leaf, stem, meristem and petiole explants to produce shoots on any of the media was not unprecedented. Endogenous hormones play a very significant role in the outcome of exogenous hormones on shoot proliferation (Band and Lineveger, 1986). Because of poor shooting responses on MS and AND media, further studies were made on GB5 medium only.

In the interaction trials it was found that the effective dilutions of NAA and BA for initiation of shoots ranged in between 0 - 1.6 ppm (Table 1).

Table 1:
Effect of NAA and BA in GB5 medium on shoot regeneration from nodes of Basella alba L. after two weeks of inoculation.

BA mg/1	NAA mg/1	Percent of culture with shoots
0 - 0.1	0 - 0.1	9.3
0.8 - 1.6	0 - 0.1	89.4
0.2 - 0.8	0.2 - 0.8	5.7

In the second experiment of interaction of NAA and BA it was found that calluses were observed in all of the treatments (Table 2).

Table 2:
Interaction of NAA and BA on callus formation.

BA in ppm	NAA in ppm					
0	0	0.1	0.2	0.4	0.8	1.6
0.1	1.2*	0.6	1.3	2.0	2.0	2.0
0.2	1.7	0.8	1.3	2.0	2.0	2.0
0.4	1.6	1.2	1.8	2.0	2.0	2.0
0.8	1.6	1.2	1.8	2.0	2.0	2.0
1.6	1.6	1.2	1.8	2.0	2.0	2.0

* x explant size

Shoots initiated only when NAA was in the range of 0 to 0.1 ppm. Higher concentrations of NAA inhibited shoot formation (Table 3).

Table 3:
Interaction of NAA and BA on shoot formation.

BA in ppm	NAA in ppm					
	0	0.1	0.2	0.4	0.8	1.6
0		2*				
0.1	3	3				
0.2	3	3				
0.4	3	3				
0.8	3	5		1		1
1.6	3	6				

* number of shoots/treatment

NAA favored root formation. Absence of BA was not critical for root formation. In some treatments, root initiation was found even in high concentrations of BA, but such incidence was rare (Table 4).

In phase II it was found that the favorable ratios for rooting and shooting were 0.1 - 0.2 ppm of BA/0.8 - 1.6 ppm of NAA, and 0.8 - 1.6 ppm of BA/0.1 - 0.2 ppm of NAA respectively. Media with the same ratios were used in phase III of this experiment. It was found that calluses did not respond to these treatments. Further studies are suggested to be conducted with the use of other hormones.

Table 4:
Interaction of NAA and BA on root formation.

BA in ppm	NAA in ppm					
	0	0.1	0.2	0.4	0.8	1.6
0			2	2	6	6
0.1			4	6	8	8
0.2			4	6	8	8
0.4		2*				
0.8				1		1
1.6						1

* number of roots/treatment

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